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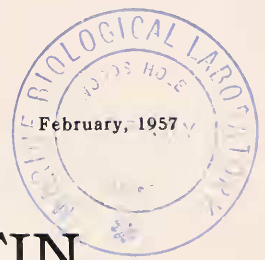

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THE DISTRIBUTION OF POLYSACCHARIDES AND BASOPHILIC SUBSTANCES DURING THE DEVELOPMENT OF THE MUSHROOM COPRINUS[1]

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As Buller (1909 *et seq.*) has pointed out, some species of the genus *Coprinus* sow their spores once during a short interval of time and the fruiting body disappears shortly thereafter by auto-digestion. Characteristically the small buds will, all in one day, go through a period of rapid expansion and elongation, shed their spores, and deliquesce. This rapidity is no doubt related to their small size, for larger species of hymenomycetes will go through many days of continued production and shedding of spores.

The origin of the gills and their individual lamellae has been described in detail by Atkinson (1916). They are formed at an early stage by the orientation of hyphae and the final result (which is illustrated in Figure 1) has a number of distinguishable component parts: the outside is covered with the hymenium which consists of a mixture of basidia and sterile paraphyses; below this there is a subhymenial layer of small hyphae; and finally the central portion of the gill lamella, the tramal layer, which is composed of large hyphae.

Borriss (1934) has shown that the development of *Coprinus* occurs in two distinct stages, one in which cell division and the initial cell orientation takes place, and it is during this stage that the gill primordia are formed. The second stage consists of rapid cell elongation and in the latter phase of this period the spores bud from the basidia. It may be inferred, from the recent results of Madelin (1956), that this period of rapid expansion involves the transfer of material from the vegetative mycelia. Such a transfer is in keeping with the views of Buller as well as with the situation in *Agaricus campestris* (Bonner, Kane and Levey, 1956).

Using histochemical techniques, it has been possible in this study to follow the distribution of certain groups of substances in the fruiting body. It could be demonstrated that these substances accumulated at specific locations in the gills and that they were all transferred into the spores, so that by the advent of auto-digestion there were virtually no demonstrable substances left within the cells at the time of the final destruction. Not only does this indicate an efficiency, an economy in the

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fruiting process, but also it is of some interest to find that prior to sporulation the different substances are stored in different parts of the gills.

Methods

Two species of *Coprinus* were studied: *C. lagopus* Fr. and *C. curtus* Kalch. (The authors would like to thank Dr. Haig P. Papazian of Yale University for the culture of *C. lagopus,* and Dr. Alexander H. Smith of the University of Michigan for the identification of *C. curtus.*) They were grown in jars on moist, sterile horse dung and kept at room temperature (approximately 24° C.)

The entire fruiting bodies were fixed for 12 hours in Rossman's fluid (9 parts absolute alcohol saturated with picric acid plus 1 part formaldehyde) at 4° C. The selection of this particular fixative, as well as the temperature, follows from the recommendations of Deane *et al.* (1946), from their studies on the preservation and localization of glycogen in mammalian liver. The suitability of the fixation for the mushroom material is evidenced by the absence of any false localization of glycogen, "glycogen flight," due to the pathways of penetration of the fixative. Following fixation the tissues were dehydrated, cleared with methyl salicylate (14 hours), and embedded in paraffin. Sections were cut at both 5 and 10 μ and mounted on slides in the usual manner.

Polysaccharides were demonstrated by the periodic acid-Schiff reaction as described by Gomori (1952). This technique is based upon the oxidation with periodic acid of the vicinal hydroxyl groups of polysaccharides to aldehyde groups and the subsequent visualization of the aldehyde groups by reaction with the Schiff reagent. The sections were deparaffinized, run to water, and oxidized for 10 minutes at room temperature with 0.5% periodic acid. Schiff reagent was prepared according to the method of Lillie (1954) and the slides were treated with it for 20 minutes. Excess Schiff reagent was removed by three sodium metabisulphite rinses, the slides dehydrated, cleared and mounted. Control sections were treated exactly the same, although not oxidized with periodic acid. Any material which stained with the PAS technique and did not stain in the control slides is hereafter referred to as polysaccharide.

It was possible to differentiate mucopolysaccharides from glycogen by digestion of the slides with salivary amylase prior to the periodic acid oxidation. The slides were run to water as before and digested for three hours at room temperature in saliva. Following digestion the slides were run through the same PAS procedure outlined above. Any material which was PAS-positive but removable by salivary digestion is hereafter referred to as glycogen. Any material not removable with salivary digestion is referred to as mucopolysaccharides. Additional slides placed in water for three hours, in place of saliva, served as control slides. Since the pattern of localization in those control slides differed in no way from the standard PAS pattern, one can conclude that the saliva removes glycogen by enzymatic hydrolysis specific for glycogen and not by a leaching-out of materials by simple dissolution in an aqueous solution.

Regions of basophilia were demonstrated in the same material by staining with a 0.1% aqueous solution of toluidin blue. Cytoplasmic basophilia can be attributed to the presence of ribose nucleoproteins and/or acid mucopolysaccharides. Since all

basophilic material observed in this study was not PAS-positive, it is assumed that the basophilia was due to ribose nucleoproteins.

Results

A series of stages of both species of *Coprinus* was fixed and sectioned. Then duplicate slides of each fruiting body were stained by (1) the PAS method for all polysaccharides, (2) the PAS method with salivary digestion for the mucopoly-

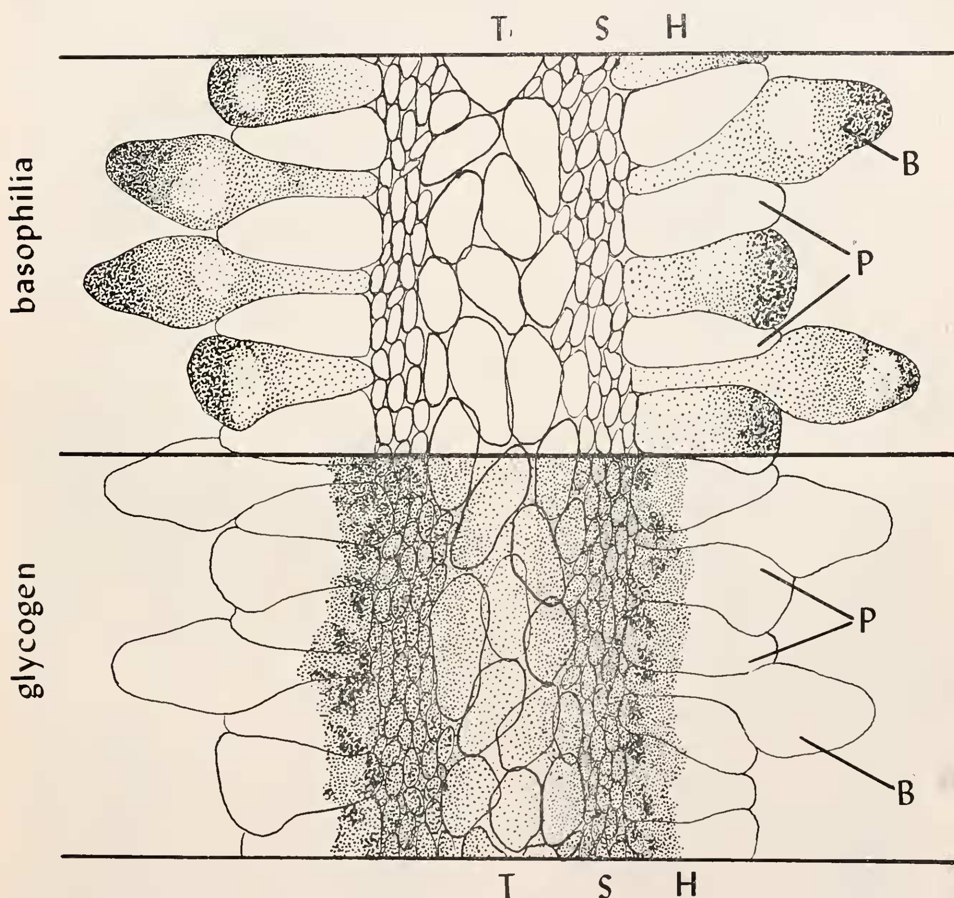

FIGURE 1. A section through a gill indicating the distribution of basophilic substances (above) and glycogen (below). T, tramal layer; S, sub-hymenium; H, hymenium; B, basidium; P, paraphysis. (Drawing by Miss Marcia J. Shaw.)

saccharides, and (3) with toluidin blue for the basophilic substances. Therefore, it was possible to compare a specific fruiting body at a specific stage for three substances: mucopolysaccharides, glycogen, and basophilic substances.

First of all it should be said that both species, *C. lagopus* and *C. curtus*, showed the same staining characteristics, and therefore the description given below applies

to both. The general staining properties of the cap and stipe could be seen more advantageously in the smaller *C. curtus,* while *C. lagopus* was especially suitable for examination of the gills.

An intense staining with Schiff reagent was observed in the globular cells of the cap in *C. curtus* which was not dependent upon periodic acid oxidation. There was also a faint staining of all cell walls in non-oxidized control sections, and character-

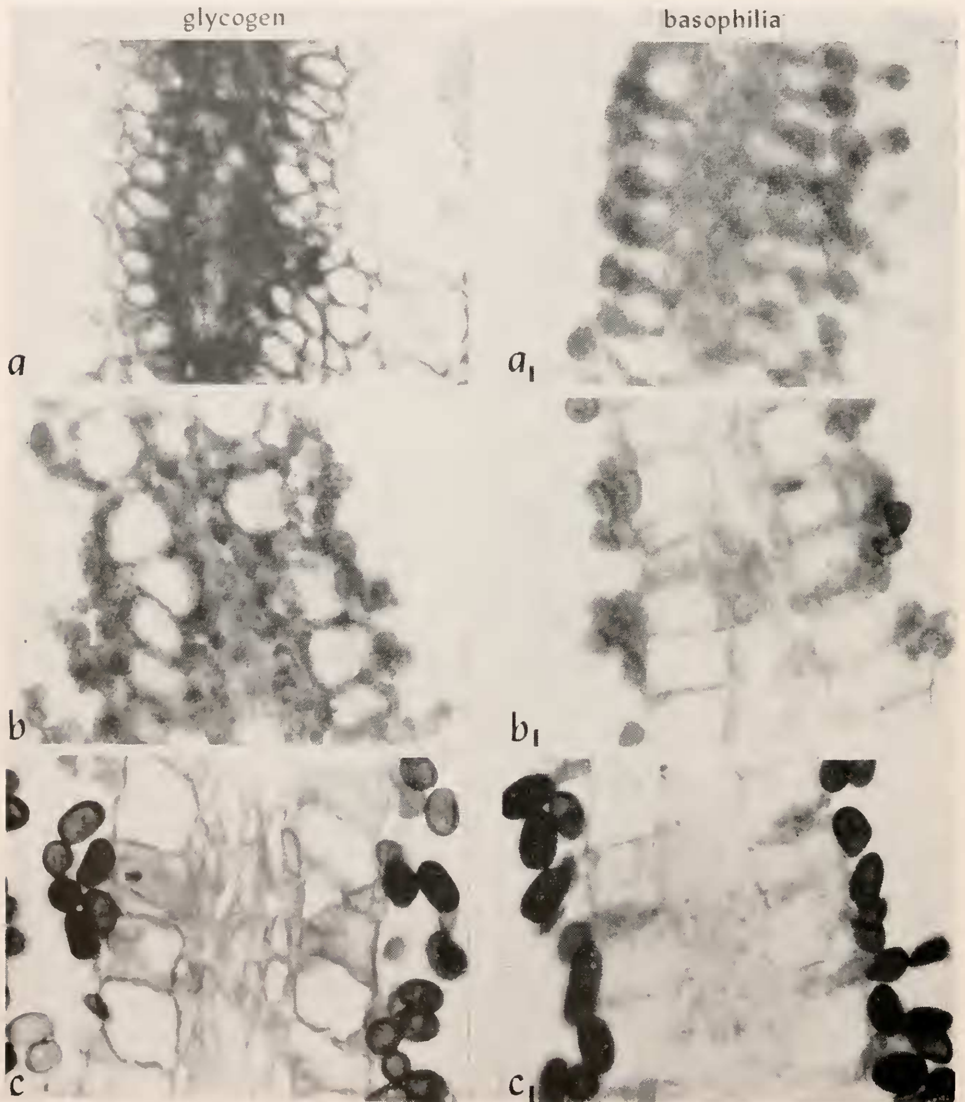

FIGURE 2. Photograph of sections through the gills showing the distribution of glycogen (left column) and basophilic substances (right column) at different stages of development; a, b, c represent the three stages of before, during, and after spore formation. Each pair (*e.g.*, a and a_1) are from the same mushroom.

istically the stain was more intense at the basal end of the mushroom. The basis for these two staining reactions is unknown. Following periodic acid oxidation the cell walls stained intensely, indicating the presence of mucopolysaccharides, presumably chitin. The cell walls were the only regions where mucopolysaccharides were observed in a significant concentration.

Exclusive of the gills, there was no general difference in the distribution of glycogen and the basophilic substances; they both appeared to be present in small quantities all over the mushroom at early stages, although the growth zone just below the cap always showed a higher concentration. The intensity of staining reactions was inversely proportional to the cell length, that is, the short cells of the growth zone at early stages were darkly stained, but after complete expansion these cells were depleted of stainable material as were the cells of the rest of the mushroom.

In the gills, prior to spore formation, there is a dark layer showing a concentration of basophilic substances, as well as a similar layer of glycogen. The interesting point is that, as shown in Figures 1 and 2, the positions of these layers do not correspond. The basophilic layer lies at the tips of the basidia, that is, the end at which the future spores will be formed. Furthermore, basophilia is observed only in the basidia and not in the paraphyses. The glycogen lies primarily in the subhymenium, although it extends to the base of the hymenium (in both the basidia and paraphyses) on one side and into the tramal layer on the other.

This staining picture is present at the earliest stages that gills are discernible, and in subsequent stages there is no obvious increase in the intensity of the staining. The only significant change is the increase in the size and the extent of the gills with age.

It was possible to show that once the spores are formed (Fig. 2, c) both the stainable layers disappear, showing a complete absence of glycogen and basophilic substances. Therefore, it is presumed that these materials enter into the spores, but the spores themselves become darkly pigmented with maturity, which masks any staining reaction they might show.

In order to verify the hypothesis that the spores contain these substances, a new series of mushrooms of intermediate size was fixed to find ones in the middle of the process of spore formation. A particular set of sections of *C. lagopus* was fortunately exactly at this stage (Fig. 2, b). The glycogen is now present in and around the hymenium, as well as concentrated in the young spores. The basophilic substances, which were at the basidial tips to begin with, appear to enter directly into the spores during their formation.

Discussion

The presence of glycogen about the base of the basidia, and its outward movement during spore formation, suggest that it might supply energy for the process of spore formation. However, a considerable portion of the glycogen enters directly into the spore and becomes part of its reserves for future germination and growth.

The basophilic substances are at the end of the basidium that forms the spores and therefore the material is transferred directly upon spore formation. Presumably the nuclear material is associated with this basophilic zone and both are transferred together.

It is of interest to note that the staining appears equally intense in the gills in

both small young mushrooms and large fully developed ones. The only difference is the extent of the gill material and from this we might presume that the gill is laid down with all its food material for sporulation right in the beginning, and during the period of growth the sole change is that the total amount of gill material is extended. From the work of Madelin (1956) it is likely that materials are constantly being drawn up from the vegetative hyphae into the mushroom during its expansion, and this material must be led directly to the newly forming gill.

The observation that essentially all stainable glycogen and basophilic substances are gone after sporulation implies an economy, and one would expect this phenomenon to be correlated with the fact that in small species of *Coprinus* there is but one short period of spore formation and discharge. Mr. Anthony J. Schmidt of this laboratory has made some preliminary PAS and toluidin blue preparations of *Agaricus campestris* and here one finds large concentrations of glycogen and basophilic substances in the cap with channels of conduction to the hymenium. Therefore in a large species which forms spores over a long period there is a large store of substances that can be continuously poured into the spores.

Summary

Two species of *Coprinus* (*C. lagopus* and *C. curtus*) were examined, using histochemical techniques, and it was found that prior to sporulation there were two distinct zones in the gills, one containing glycogen and one containing basophilic substances. The glycogen zone is at the base of the hymenium, extending into the central tramal layer. The basophilic zone is at the outer tips of the basidia. Upon sporulation both these groups of substances entered the spores, leaving no demonstrable material within the cells of the gills.

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